DILATATION OF ARTERIOLES OF THE FROG SUBMAXILLARY MUSCLE UNDER THE INFLUENCE OF VISIBLE LIGHT

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Visible light, as usually used for intravital microscopy, causes dilatation of the arterioles of the denervated frog submaxillary muscle. The minimal effective intensity of illumination of the object was 15 lx. The degree of dilatation of the arterioles in response to maximal intensity of light (250 lx) was inversely proportional to the initial diameter of the vessel. Arterioles 20-40  $\mu$  in diameter differed in their reactivity depending on the thickness of their walls. Highly reactive arterioles have thick walls. Their diameters were doubled or more by illumination with visible light. Photic vasodilatation is reversible.

KEY WORDS: arterioles; vasodilatation; microcirculation; effect of light.

Some of the methods used for intravital study of microvessels, especially those involving photographic recording, require intensive illumination. It is assumed that the light has no appreciable effect on vascular

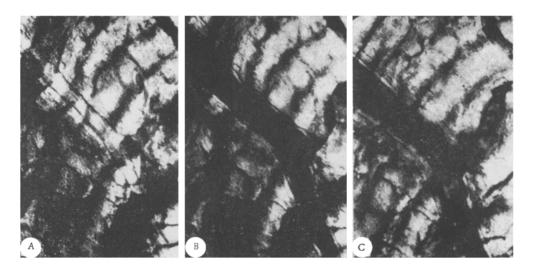


Fig. 1. Dilatation of arteriole of frog submaxillary muscle (initial diameter 20  $\mu$ ) during illumination with visible light (250 lx); A) state of arteriole with illuminator switched on: vessel wall strongly contracted and thick; B) 3 min 35 sec later: arteriole unevenly relaxed, bead-like constrictions formed along the course of the vessel; C) 15 min later: diameter of vessel increased by 170%. Objective 9×, camera objective 20 ×.

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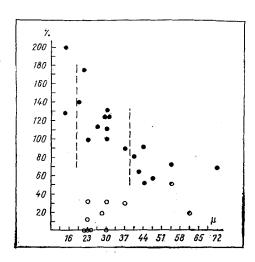


Fig. 2. Degree of dilatation of arterioles depending on initial diameter of vessels. Vessels  $20-40~\mu$  in diameter, among which a group of highly reactive (filled circles) and poorly reactive (empty circles) vessels can be distinguished, lie between broken lines; half-filled circles denote experiments under urethane. Abscissa, diameter of vessels (in  $\mu$ ); ordinate, increase in diameter (in %), relative to initial value taken as 100%.

tone under these conditions. However, Finsen [5] showed that human cutaneous vessels are sensitive to light and later, in his laboratory dilatation of all the vessels of the frog tongue and stasis in the capillaries were observed under the influence of light. This response was ascribed mainly to the action of ultraviolet radiation. No attention has been paid to these observations, probably because visible light is usually used for observations on microvessels.

The effect of visible light on the microvessels of frog skeletal muscle is described in this paper.

## EXPERIMENTAL METHOD

The submaxillary muscle was exposed in frogs (Rana temporaria), anesthetized with Viadril (3.7 mg, intravenously), by the method described by Mirzadaeva [1]. Branches of the trigeminal and facial nerves supplying this muscle [2] were divided. The muscle was kept constantly moist with Ringer's solution. The frog was placed on the stage under an MBI-6 microscope. Observations were made in transmitted light from a specially constructed illuminator [3]. Light from the source (a 90 W, 12 V incandescent lamp) passed through a heat filter (5% solution of CuCl<sub>2</sub>, thickness of the layer of solution 20 mm) and also through a green glass filter (main

transmission band 30% in the region  $\lambda=490\text{-}620$  nm). The lantern and lamp of the illuminator were cooled by a fan. The temperature of the muscle in the region of the light spot was measured by a differential thermocouple (Chromel-Copel). One of its junctions was placed on the dorsal surface of the muscle, the other in a U-3 ultrathermostat. The difference in thermoelectromotive force was measured with an F-18 amplifier.

The state of the vessels under different intensities of illumination was recorded by serial photography. Exposure to light of each intensity did not exceed 15 min. Usually the experiment began with minimal intensity of illumination; According to readings of the light meter, in the plane of the object it was 15 lx. The intensity was then increased stepwise. Maximal intensity of illumination with the nominal voltage to the illuminator lamp was 250 lx. The illuminator was switched off for 15-60 min between exposures. The results given below are pooled data for 33 vessels obtained by measurement of serial photomicrographs. Only those experiments in which the mean pressure in the dorsal aorta was not below 15 mm Hg were included in the analysis. The pressure was measured by an electromanometer and recorded on the KSP-4 potentiometer.

## EXPERIMENTAL RESULTS

Visible light had a powerful effect on the arterioles of the denervated skeletal muscle and lowered their tone. During the first few seconds of exposure the blood vessel wall could be seen to be strongly and unevenly contracted. Bead-shaped constrictions were seen on the arteriole (Fig. 1A). Under the influence of light the smooth muscles of the vessels started to relax (Fig. 1B) and the walls of the vessel became straight and smooth (Fig. 1C). The general pattern of the blood flow in the microcirculation was substantially modified. After a few seconds, observation in bright light revealed that all the vessels were dilated and the blood flow greatly increased.

With the nominal voltage on the illuminator lamp and, correspondingly, the maximal photic flux, the temperature in the region of the light spot was increased by not more than 0.1°C, even in the absence of the fan. This amount does not exceed the ordinary fluctuations of the air temperature in the experimental room. Consequently, the changes observed in these experiments in vascular tone were not due to heating of the muscle in the spot of light.

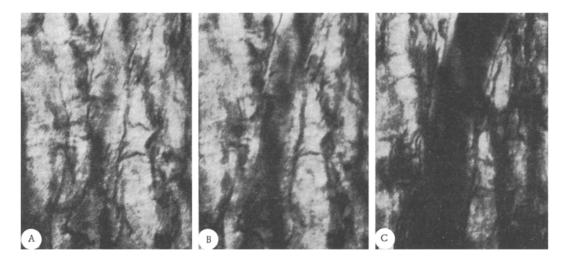


Fig. 3. Effect of visible light (250 lx) on arteriole with low reactivity (diameter 20  $\mu$ ): A) state of arteriole when light switched on: thin walls of vessel can be seen; B) 1 min later: diameter of vessel unchanged, blood flow slightly increased; C) 15 min later: diameter increased by 30%, marked increase in blood flow, as shown by dark color of vessel on photomicrograph.

The intensity of photic vasodilatation differed with the diameter of the vessel. The diameter of capillaries measuring 12-13  $\mu$  was unchanged by light even of maximal intensity (250 lx). However, the blood flow in them was considerably accelerated. Movement of blood began to take place along capillaries in which no flow had existed before switching on the light.

It will be clear from Fig. 2 that the smaller the initial diameter of the arteriole, the more it was increased by light. However, some vessels did not obey this rule. This group included nine vessels with an initial diameter of 20-40  $\mu$  (see Fig. 2, empty circles). The diameter of four vessels was unchanged, even by light of maximal intensity (250 lx). The remaining five vessels of this group were dilated only by 13-33%, whereas 10 other vessels with the same initial diameter (this group lies between the broken lines in Fig. 2) were dilated by 90-175%.

Arterioles belonging to groups with low and high reactivities to visible lightdiffered in the structures of their walls. The walls of arterioles with low reactivity were thin. They were seen on the photomicrographs as dark lines bounding the vessels (Fig. 3). From their order in the vascular network these vessels evidently belonged to the class of metarterioles or precapillary sphincters, in the terminology of Zweifach and Chambers [8]. The walls of the highly reactive arterioles could be seen on the photomicrographs as much thicker bands (Fig. 1A). These vessels with thick walls could be classified as terminal arterioles [8].

Small arteriolar branches given off by reactive arterioles were strongly dilated (by 130-200%) by light, although their actual walls could be quite thin and their initial diameters were similar to those of capillaries (under  $20\,\mu$ ). Arterioles with larger external diameters ( $40-70\,\mu$ ) also were dilated by light, but by not more than 90% compared with initially (Fig. 2). Photic vasodilatation is reversible. Constriction took place slowly in darkness. After illumination for 10-15 min, usually a stay of at least 1 h in darkness was necessary before the diameter of the vessel returned to its initial value.

The minimal effective intensity of light was 15 lx. In light of this intensity vessels began to dilate after 3-6 min. With an intensity of 250 lx the latent period of response was 4-5 sec. Visually the beginning of the response could easily be detected by the more rapid motion of the red blood cells. With an intensity of illumination of 250 lx, by the end of the first minute the diameter of the vessel had increased to 92-95% of the maximal dilatation, which was usually reached after 10-15 min.

The effect of light on smooth muscles of the internal organs and vessels was studied particularly intensively in the 1920's [4, 7]. As a rule, the workers concerned found that ultraviolet light was highly effective whereas visible light was effective only after preliminary sensitization by various substances. It was therefore necessary to find out whether Viadril possesses photosensitizing properties of this sort. In a control experiment a frog was anesthetized with urethane (180 mg, intravenously) and anesthesia was maintained with ether, whereas in the second experiment urethane (100 mg, intravenously) was combined

with tubocurarine (0.2 mg, intravenously). The arterioles of both frogs were sensitive to the visible part of the spectrum and their responses were indistinguishable from those described above (Fig. 2).

It is not only frogs whose blood vessels are sensitive to visible light. Furchgott and co-workers [6] found relaxation of a strip of rabbit aorta under the influence of visible light. However, responses of the microvessels to illumination with visible light, so far as the writer knows, have not previously been described. Nevertheless, photic vasodilatation changes the state of the microvessels so strongly that it must certainly be taken into consideration during intravital microscopy of blood vessels, at least in frogs.

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